INFRARED STUDIES OF WHEAT GLUTEN

by

GORDON WILLIAM DUEKER

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INTRODUCTION

It is generally recognized that the inherent baking quality of wheat flour is determined by the quality as well as by the quantity of its gluten. Although the quantity of protein can be determined by Kjeldahl analyses, no completely satisfactory method has been developed to measure gluten quality. The sedimentation test developed by Zeleny (1) is a valuable physical approach to this problem. However, the greatest need still exists for microphysical and microchemical methods suitable for application to the small samples available in the wheat breeding program.

Attempts to relate baking quality to the amino acid content of the gluten have met with mixed success. The investigations of Pence et al (2) employing glutens washed from 17 flours of widely differing types and sources showed no significant differences in amino acid content for any of the flours evaluated. Wöstmann (3), however, found that the polarographically determined cystine content of wheat flour proteins was positively correlated with loaf volume. The correlation coefficient between protein content of flour and extensogram area was +.82, between cystine content of flour protein and extensogram area +.77 and between total cystine content of flour and extensogram area was +.92. Miller et al (4) reported a significant difference in the percent cystine for wheat grown in two different crop years. Differences in both the methionine and cystine content also were noted between the same varieties of wheat grown

in different districts. A positive correlation was noted between dough mixing time and percent cystine as influenced by environment.

Further evidence of the effects of sulfur on gluten strength is afforded by the work of Pietz (5) and Sullivan et al (6). The work reported by the latter authors explains the action of oxidizing and reducing agents on dough in terms of their effect on the sulfur linkages of gluten proteins. Pietz (5) found that good gluten, having a swelling number of 17, had a nitrogen to sulfur ratio of 1 to 0.0295, while for a weak gluten, having a swelling value of 1, the nitrogen to sulfur ratio was 1 to 0.0158.

The sensitivity of the infrared spectrum of a substance to slight changes in molecular structure suggested that the quality variations among wheat glutens might be paralleled by recognizable differences in their spectra. The present study was initiated to develop a suitable technic for studying the infrared spectrum of flour protein and to apply that technic to a study of flour samples of varying quality.

MATERIALS AND METHODS

One series of samples was milled from wheat collected for the Western Regional Research Laboratory's investigations of baking quality (7). Origin of the samples and some measurements made by the Western Regional Research Laboratory are summarized in Table 1. Other samples were selected from those wheats which have been evaluated by the Federal Hard Wheat Quality Laboratory.

Gluten was obtained from these samples by a suitable technic (8).

Table 1. Characteristics of flours from which glutens were extracted.

Class	Variety	Origin	Protein flour	Protein gluten	Loaf volume	Specific Sedimentation
			percent		cc	
White	Baart	Wash.	9.3	85.5	1215	----
SRW	Goens	Indiana	9.0	85.9	1245	3.7
HRW	Turkey	Kansas	13.2	83.8	1360	5.6
HRW	Red Chief	Kansas	10.2	81.6	1000	3.1
HRS	Premier	N. Dak.	11.5	84.3	1205	3.4
Red Durum	Pentad	Dakotas	11.4	77.1	690	2.6

Initial attempts to observe the spectrum of gluten samples were made using films evaporated on plates of silver chloride. A 10 percent sodium salicylate solution was tried as a suspending medium for the gluten, but was discarded when it appeared that coagulation of the gluten occurred upon evaporation, making a film with a granular structure which was relatively opaque to infrared radiation. The most satisfactory films were prepared by placing a small amount of the gluten suspended in 0.1 N acetic acid on a plate and evaporating the suspending medium over a sodium hydroxide solution (10 percent relative humidity). In order to obtain thicker films this process was repeated as often as necessary.

The preparation of gluten films was abandoned in favor of the mull technic, which has been found to be satisfactory both

from the standpoint of maintaining the gluten in its original condition and obtaining samples of sufficient infrared transparency. This method has the additional advantage of providing better control over the amount of material placed in the radiation beam than is possible using films evaporated from solution. The preparation of the mull involved mixing a weighed amount of 60-mesh dry gluten powder with a weighed amount of Nujol in a mortar for a few minutes. A drop of this mixture was placed on a rock salt plate on which a metal spacer was laid. Another rock salt plate was placed on top and the plates were clamped together with a brass holder. It was found using this method that for sufficient concentration of gluten in the mull, the sample was too opaque to permit accurate measurements of the optical density, even when cells of capillary thickness were used. The gluten in the mull also tended to settle out as soon as stirring was stopped, so that it was difficult to obtain samples of consistent concentration. Since it was thought that the opacity of the samples was caused by the relatively large size of the particles of gluten, a small ball-mill using glass balls was constructed, and the samples milled until the particle size was considerably reduced. The optimum time of milling appeared to be 48 hours, as evidenced by the tendency for the gluten to adhere to the walls of the ball mill. However, it was noted that different samples of gluten took different lengths of time to be reduced to the same state of division.

Ball milling of the gluten enabled mulls to be made which

were considerably less opaque than the unmilled samples. Trouble was still encountered due to the settling of the powder in the oil. In order to alleviate this difficulty a substance of higher viscosity was sought. The most satisfactory vehicle was vaseline, which exhibits a spectrum quite similar to that of Nujol except for two additional bands at 13.67 and 13.81 microns which do not appear in Nujol. Since no absorptions in the gluten were found beyond 9.6 microns, this did not offer any difficulties.

Measurement of the optical density of gluten in the region of 3.43 and 3.51 microns was inaccurate because of the intense C-H absorption bands of the vaseline at these positions. In order to make accurate measurements in this region a vehicle was needed which had no C-H absorption. The fluorine-substituted hydrocarbons are suitable for this purpose, and are reasonably transparent out to 5.7 microns. The material used in this investigation was perfluro-lubricant FCX-512, manufactured by the Du Pont Chemical Company.

Spectra of the samples were run from 2.5 to 14.0 microns on a Perkin-Elmer Model 12-B spectrometer modified for automatic recording by the addition of a General Motors breaker amplifier, and a Leeds and Northrup Speedomax recorder. In this instrument the infrared radiation passes through the sample cell, is dispersed by a rock salt prism, and a narrow band of the spectrum thus produced is focused on a thermocouple. A block diagram (Fig. 1) shows the arrangement of the recording system. The thermocouple output is amplified sufficiently by the breaker amplifier (A) to operate the recording potentiometer (B). The

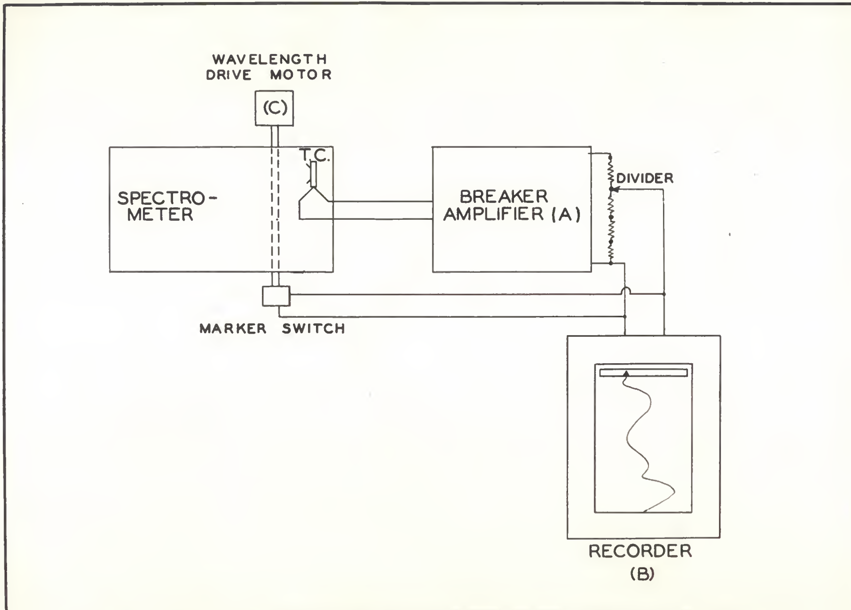

Fig. 1. Schematic diagram of spectrometer recording system.

wavelength drum of the spectrometer is driven by a motor (C) in step with the chart of the potentiometer. Thus the potentiometer plots the transmitted intensity vs. drum setting. A cam on the wavelength drive shaft closes a switch every one-tenth revolution causing fiduciary marks to be produced on the recording.

To compensate for absorption of the vehicle used in the mull, two curves were run on the same chart, one recording the deflection when a mull of gluten and vaseline was in the beam, and the other, the deflection of the potentiometer when a cell containing pure vaseline was in the beam. The two curves were aligned by means of the reference marks and optical density (see Appendix, p. iii) could thus be calculated for any wavelength directly from the chart.

Despite the improvement in transmission of the samples due to ball milling, a sample of sufficient concentration to show more than the most intense absorption bands proved to have an optical density too high to measure with any degree of accuracy. Therefore a divider was constructed to reduce the deflection when the background absorption curve was being run, and allow larger deflection for the sample. This divider consisted of four precision resistors each having the same value of resistance. The four resistors were connected in series across the output of the breaker amplifier, as shown in Fig. 1. By means of a tap switch, connection could be made to feed either all, 3/4, 1/2, or 1/4 of the output of the amplifier into the recorder. When calculating the optical density of a curve run using this device, it was only necessary to add a constant factor depending upon

the fraction at which the divider was set.

Examination of a number of measurements on a single sample of gluten showed considerable variation in optical density from curve to curve. This variation was thought to be due to the difficulty of reproducing cell thickness using the demountable cell holder described previously. A cell was constructed which could be filled and samples changed without dismantling. This consisted of two rock salt plates clamped securely together between 1/4" brass plates. The salt plates were separated by a 0.1 mm aluminum spacer, which was formed in two pieces to allow entrance and exit passages. A channel drilled in the entrance side of the spacer allowed insertion of a hypodermic needle, which was sealed in place with laboratory wax. By use of a hypodermic syringe, the cell could be filled with the mull of gluten and vaseline, or emptied by flushing with a solvent. This cell was used for all measurements beyond 6.6 microns. However, in the region below 6.6 microns, the demountable cell was used, because a thinner spacer was necessary, and because of the difficulty of finding a suitable solvent for the perfluro-lubricant used in this region.

RESULTS AND DISCUSSION

Amino Acid Spectra

The spectra of dl-methionine, l-cystine, l-cysteine hydrochloride, and a polypeptide, glutathione, are shown in Figs. 2 and 3. Samples were prepared by mulling 0.5 g of the

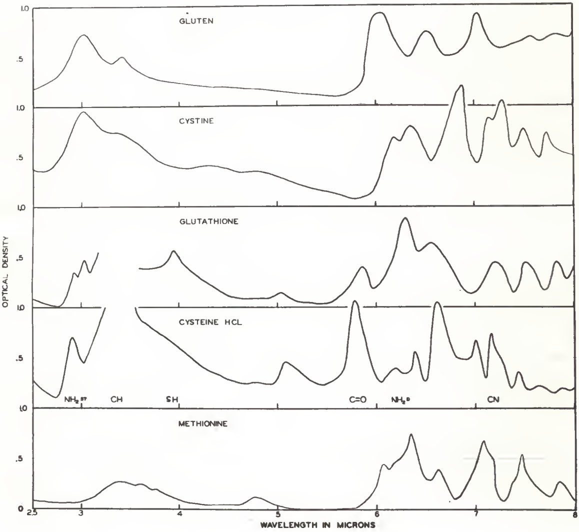

Fig. 2. Infrared spectra of methionine, cysteine hydrochloride, glutathione, cystine, and a representative gluten, from 2.5 to 8 microns.

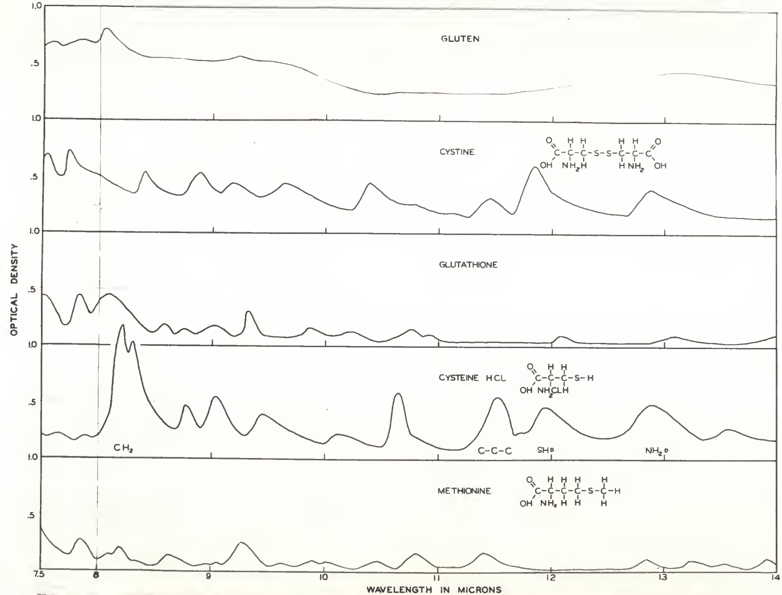

Fig. 3. Infrared spectra of methionine, cysteine hydrochloride, glutathione, cystine, and a representative gluten, from 7.5 to 14 microns.

crystalline substance in 25 drops of Nujol. The spectrum of cystine is in good agreement with that published by Wright (9), and can be distinguished from the spectrum of dl-cystine. The spectrum of cysteine hydrochloride from 2.5 to 9.1 microns agrees closely with that given by Randall et al (10).

The S-H stretching frequency, which would be expected (11) to cause absorption at 3.93 microns was not observed in the cysteine hydrochloride, but a band of moderate intensity appears at this position in the glutathione. It had been hoped that an indication of the sulfur content of the gluten samples could be obtained using this absorption, but the low intensity of the band, even in samples in which the concentration of the linkage is high, would seem to rule out this possibility.

Gluten Spectra

The spectra of samples of gluten treated with oxidizing and reducing agents were run from 2.5 to 14 microns. One percent gluten solutions in 0.1 N acetic acid were treated with 0.11 mg of sodium sulfite or 0.125 mg of potassium iodate and films were prepared from both treated and untreated glutens. These treatments caused no change in position of any of the absorption bands. A set of samples treated with five times as much oxidizing or reducing agent was prepared. Again no difference was observed between the spectra of the treated and untreated samples. It could not be determined if there was any change in intensity of the absorptions observed, because of the difficulty of preparing solid films of constant thickness. For this reason further in-

vestigation was limited to samples prepared by the mull technic.

The spectra of the glutens described in Table 1 are shown in Figs. 4, 5, 6 and 7, and one curve is included in Figs. 2 and 3 for comparison with the spectra of pure amino acids. It is apparent that few of the absorptions of the pure amino acids appear at the same positions in the gluten. At the S-H stretching frequency there is no indication of an absorption band, and although a difference in the absorption exists, it is not large enough to be statistically significant. No absorptions were observed in the gluten samples from 11.0 to 14.0 microns.

Tentative assignments of the various absorption bands are given in Table 2, together with the specific material on the spectra of which the assignment was based. In Table 3, the optical densities of the glutens are listed, together with numbers indicating their relative optical densities at the absorption bands recorded in Table 2. Considerable difference in optical density exists among glutens at the 3.03, 3.42, 6.08, and 6.52 micron absorptions. These differences remain if the N-H absorption at 3.03 microns is adjusted to be proportional to the protein content of the sample. Although the differences between samples beyond 7 microns are not statistically significant at the 5 percent level, it is possible that further measurements in this region would reveal definite differences. The major causes of error in measurements, including cell thickness and sample concentration, are such as to cause a constant error in the same direction over the entire region of the spectrum. Thus if the optical densities of all the samples are adjusted to be

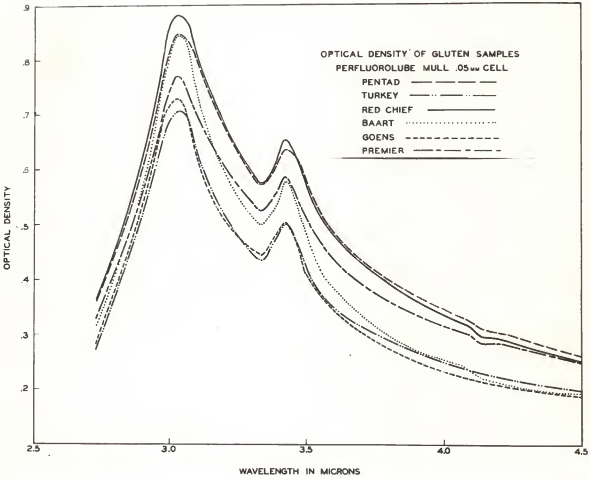

Fig. 4. Infrared spectra of gluten samples from 2.5 to 4.5 microns.

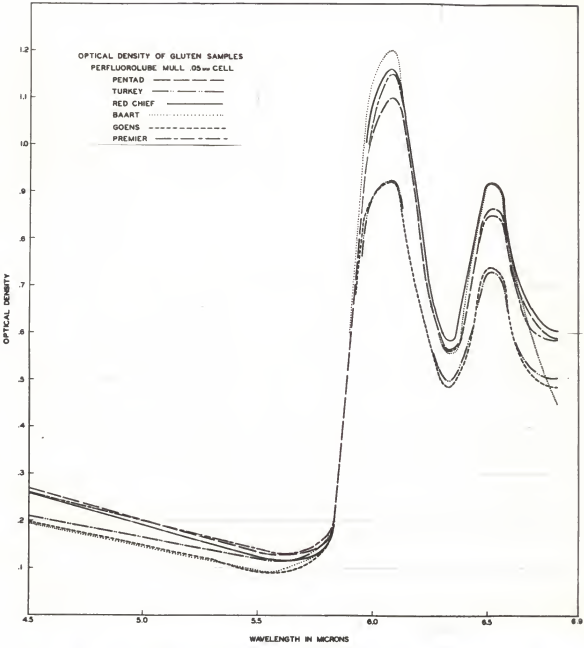

Fig. 5. Infrared spectra of gluten samples from 4.5 to 6.9 microns.

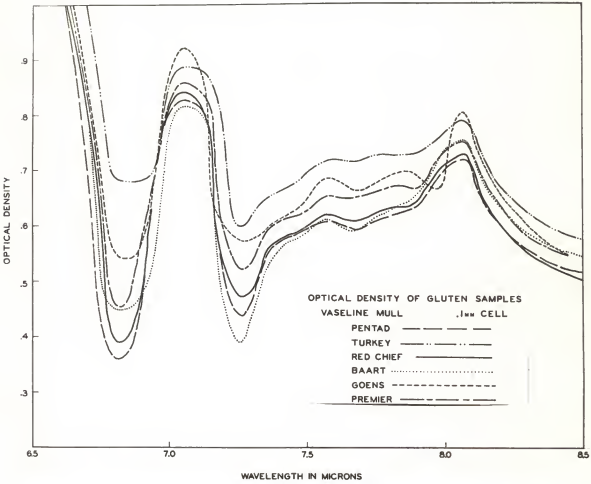

Fig. 6. Infrared spectra of gluten samples from 6.5 to 8.5 microns.

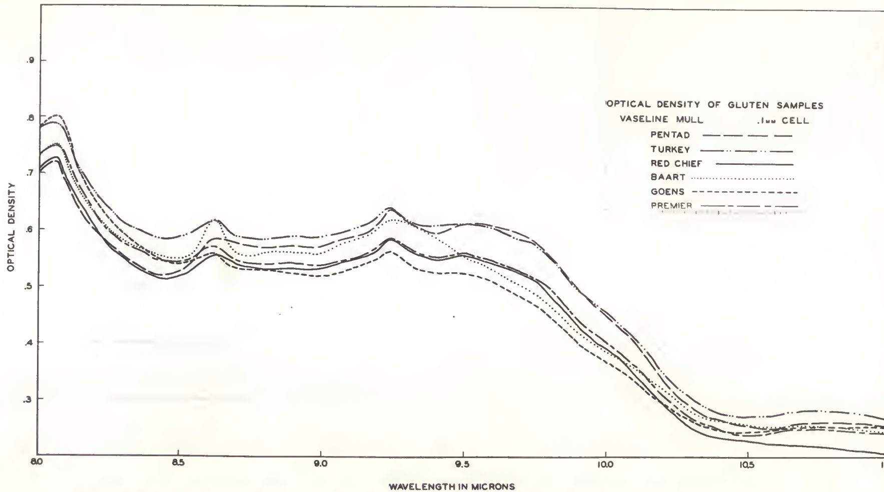

Fig. 7. Infrared spectra of gluten samples from 8.0 to 11.0 microns.

Table 2. Tentative assignments of infrared absorption in gluten.

Wavelength microns	Assignment	Ref.	Substance analyzed and wavelength of band
3.03	Amide N-H stretching	(12)	Tyrocidine (3.05 μ)
3.42	C-H stretching	(12)	Tyrocidine (3.30 μ)
6.08	C=O stretching	(12)	Bovine serum albumin (6.05 μ)
6.52	N-H bending	(12)	Salmine (6.45 μ)
7.05	C-H bending	(12)	Cytochrome c (7.05 μ)
7.58	Unassigned		
8.06	C-O stretching	(13)	Glycine (8.00 μ)
8.62	C-H bending	(12)	Bovine serum albumin (8.60 μ)
9.24	C-OH stretching	(12)	Salmine (9.30 μ)
9.50	Unassigned		

Table 3. Optical density of various gluten samples.

Variety	Wavelength μ										
	3.03	3.42	4.08	6.08	6.52	7.05	7.58	8.06	8.62	9.24	9.50
Red Chief	$_{1}\underline{.881}$*	$_{1}\underline{.654}$*	.317	$\underline{1.16}_{1}$*	$_{1}\underline{.885}$*	.842	.620	.727	.588	.587	.559
Baart	$_{1}\underline{.848}$	$_{6}\underline{.578}$	.241	$\underline{1.22}_{1}$**	$_{1}\underline{.875}$*	.814	.612	.751	.620	.620	.558
Pentad	$_{1}\underline{.848}$	$_{1}\underline{.637}$*	.329	$\underline{1.10}_{1}$*	$_{1}\underline{.845}$*	.827	.608	.720	.586	.640	.614
Premier	$_{6}\underline{.771}$	$_{1}\underline{.587}$	.305	$\underline{1.15}_{1}$	$_{6}\underline{.825}$	.858	.654	.750	.571	.586	.561
Goens	$_{6}\underline{.729}$	$_{6}\underline{.504}$	.226	$_{6}\underline{.920}$	$_{6}\underline{.705}$	.918	.686	.803	.559	.564	.526
Turkey	$_{6}\underline{.706}$	$_{6}\underline{.500}$	.241	$_{6}\underline{.923}$	$_{6}\underline{.702}$	.887	.719	.790	.620	.642	.619
	Difference at the five percent level										
	.122	.122	.122	.122	.122	.198	.198	.198	.198	.198	.198
	Difference at the one percent level										
	.282	.282	.282	.282	.282	.479	.479	.479	.479	.479	.479

* Significance exceeds five percent level.
** Significance exceeds one percent level.

the same at 7.58 microns, the differences at 9.50 microns, instead of becoming less, as might be expected if they are due to these causes, actually become greater.

A noticeable difference in the appearance of the curves exists at about 9.50 microns. In this region, Pentad and Turkey glutens appear to have an absorption which is not present in the other samples. Two other glutens, described in Table 4, were found to differ in this same manner, with Kharkof having the higher, and Chiefkan the lower optical density at 9.50 microns. Their densities were the same at 10.23 and 9.4 microns. The maximum difference in optical density between these two was .1.

Table 4. Description of gluten samples having different optical densities at 9.50 microns.

Variety	Protein percent	Location grown	Loaf volume cc
Kharkof	16.6	North Platte	881
Chiefkan	16.7	North Platte	768

Based upon the data in Table 2 and the curves presented in Figs. 4, 5, 6 and 7, it is probable that differences in the spectra of glutens do exist. However, since differences are small, great care must be taken in preparing samples. In any series of samples the quantity of gluten should be adjusted to give the same equivalent protein concentration in each sample. This would decrease variations of optical density due to this factor and make more evident those due to quality differences.

A factor which was not taken into account in this work, but

which must be considered if precise quantitative measurements are to be made is the amount of radiation scattered from the particles of the sample. This scattering varies with the volume, shape, and index of refraction of the particles, and with the wavelength of the radiation. It was assumed in this work that the particles of the various samples were identical and that effects from this cause could be ignored.

SUMMARY

Spectra of several amino acids, and samples of gluten washed from flours of widely differing characteristics were run from 2.5 to 14 microns. Assignments of some of the absorption bands in the gluten are given, and the differences of the spectra among glutens are noted. It was concluded that differences do exist among the spectra of glutens from various flours, although further investigation is required before any statement can be made relating these differences in spectra to differences in the baking quality of the flours.

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APPENDIX

ORIGIN OF INFRARED SPECTRA

Atoms of a molecular substance are bound together by forces of an elastic nature. Thus when the molecules are disturbed by external forces, such as electromagnetic radiation, the atoms vibrate about their equilibrium positions with frequencies which have been found to lie in the neighborhood of 10^{13} to 10^{14} per second. Since these frequencies are of the same order of magnitude as those of infrared radiations some interaction might be expected between the two. It is found that those molecular vibrations which produce a change in the dipole moment absorb radiation of the same frequency as the vibration.

The vibrations of simple molecules can be accurately predicted from theoretical considerations if the molecular dimensions and inter-atomic forces are known. However, in the case of complex molecules the mathematical treatment becomes prohibitively difficult.

In spite of the difficulty of predicting completely the vibrations of a molecule, much information can be gained from the observed, and theoretically explained fact that certain atomic linkages, such as C-H, N-H, etc., always vibrate at approximately the same frequency, regardless of the rest of the molecule to which they are attached. Thus the presence of such linkages may reasonably be inferred from the presence of the corresponding absorption.

In order that a particular atomic linkage give rise to an infrared absorption at a frequency which is substantially constant

from molecule to molecule, it is necessary that either the bond strength or the masses involved be considerably different from those in the rest of the molecule. Thus, vibrations involving hydrogen, or heavy elements such as sulfur, phosphorus, and the halogens, and those involving double and triple bonds, are likely to occur within narrow ranges of wavelength.

On the other hand, vibrations involving C-C bonds, C-O, C-N, and the like, where the constituents are of similar mass, are extremely subject to shifting due to adjacent elements, and thus the complete spectrum of a compound furnishes an identification which is very sensitive to changes in molecular structure.

The relationship between incident and transmitted radiation traversing an absorbing medium is Lambert-Beer's law. This is stated as

$$I = I_0 e^{-kcx}$$

Where I_0 is the intensity of the incident radiation

I is the intensity after traversing a distance x

k is a constant dependent upon the wavelength and the material

c is the concentration of the absorbing medium

This relationship has been shown to hold reasonably well in the infrared region of the spectrum in the absence of intermolecular forces.

By taking the logarithm of the above expression:

$$\log \frac{I_0}{I} = kcx$$

a quantity known as the optical density for a particular wavelength is obtained. If two or more absorbing materials are present in

the beam the total optical density is the sum of the individual optical densities.

$$D_t = k_1c_1x_1 + k_2c_2x_2 \qquad (1)$$

To obtain D_t it is necessary to pass a known intensity of radiation I_o through a sample and measure the transmitted intensity I. In measuring the optical density of a liquid or gas the transmitted intensity with an empty cell in the beam can be used as I_o. In measuring the absorption of a dissolved substance, or mixture, it is necessary to take into account the absorption of the solvent or vehicle. The method by which this is done is shown in Fig. 8, where I_o is the incident radiation, I_1 the amount transmitted by the vehicle, and I_2 that transmitted by the vehicle and sample together. Thus the optical density, D_v, of the vehicle is

$$\log \frac{I_o}{I_1}$$

and optical density of the sample plus vehicle, D_m, is

$$\log \frac{I_o}{I_2}$$

but from (1), the optical density due to the sample alone, D_s, is given by

$$\begin{aligned} D_s &= D_m - D_v \\ &= \log \frac{I_o}{I_2} - \log \frac{I_o}{I_1} \\ &= \log \frac{I_1}{I_2} \end{aligned}$$

Thus, it is possible to measure the intensity transmitted by the vehicle alone and use it as the incident radiation.

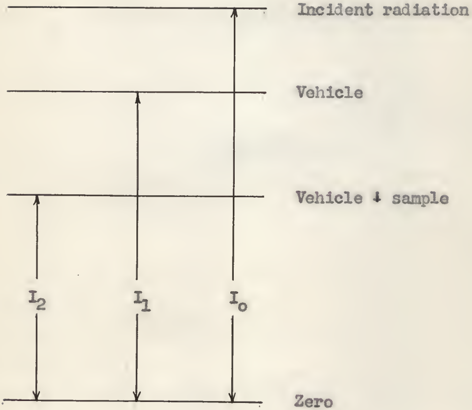

Fig. 8. Method of measurement of optical density of a mull.

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Samples of gluten obtained from flours of widely varying baking characteristics were subjected to infrared analysis. A technic for obtaining the spectra of dry gluten powders was developed. Spectra of the samples were given from 2.5 to 11.0 microns, in which statistically significant differences in the absorption were observed at 3.03 microns, 3.42 microns, 6.08 microns and 6.52 microns, and an apparent difference at 9.50 microns. Tentative assignments for some of the absorption bands were given.

It was concluded that differences do exist among the spectra of glutens from various flours, although further investigation was required before any statement would be made relating these differences in spectra to differences in the baking quality of the flours.